

AMENDMENTS

Listing of Claims:

1. (original) A method for isolating small RNA molecules from cells comprising:
 - a) lysing the cells with a lysing solution to produce a lysate;
 - b) adding an alcohol solution to the lysate;
 - c) applying the lysate to a solid support;
 - d) eluting small RNA molecules from the solid support; and,
 - e) using or characterizing the small RNA molecules.
2. (currently amended) The method of claim 1, wherein the small RNA molecules include miRNA, siRNA, snRNA, snoRNA, [[and/or]] tRNA molecules, or combinations thereof.
3. (original) The method of claim 2, wherein the small RNA molecules are miRNA molecules.
4. (original) The method of claim 1, wherein at least 20% of the small RNA molecules from the cells are isolated.
5. (original) The method of claim 4, wherein at least 50% of the small RNA molecules from the cells are isolated.
6. (original) The method of claim 1, wherein the lysing solution comprises a chaotropic agent or detergent.
7. (original) The method of claim 6, wherein the lysing solution comprises a chaotropic agent.
8. (original) The method of claim 7, wherein the concentration of the chaotropic agent in the lysing solution is at least about 2.0 M.
9. (original) The method of claim 7, wherein the lysing solution comprises guanidinium.

10. (original) The method of claim 9, wherein the concentration of guanidinium is at least about 2.0 M.
11. (original) The method of claim 10, wherein the lysing solution further comprises a detergent and a buffer.
12. (original) The method of claim 11, wherein the concentration of the detergent is about 0.1% to about 2%.
13. (original) The method of claim 12, wherein the detergent is N-lauroyl sarcosine.
14. (original) The method of claim 11, wherein the concentration of the buffer is about 10 mM to about 300 mM.
15. (original) The method of claim 1, further comprising extracting small RNA molecules from the lysate with an extraction solution comprising an organic solvent prior to applying the lysate to the solid support.
16. (original) The method of claim 15, wherein the extraction solution comprises phenol.
17. (original) The method of claim 16, wherein the extraction solution further comprises chloroform.
18. (original) The method of claim 1, wherein the amount of alcohol solution added to the lysate makes the lysate about 20% to about 70% alcohol.
19. (original) The method of claim 18, wherein the amount of alcohol solution added to the lysate makes the lysate about 50% to 60% alcohol.
20. (original) The method of claim 18, wherein the alcohol solution is added to the lysate before extraction with an organic solvent.
21. (original) The method of claim 1, further comprising washing the solid support with a first wash solution after applying the lysate to the solid support.

22. (original) The method of claim 21, wherein the first wash solution comprises a chaotropic agent.

23. (original) The method of claim 22, wherein the chaotropic agent is guanidinium and the first wash solution further comprises alcohol.

24. (original) The method of claim 21, further comprising washing the solid support with a second wash solution after washing with the first wash solution.

25. (original) The method of claim 24, wherein the second wash solution comprises alcohol.

26. (original) The method of claim 1, wherein the small RNA molecules are eluted from the solid support at a temperature of about 60 °C to about 100 °C.

27. (original) The method of claim 1, wherein the small RNA molecules are eluted from the solid support with a low-ionic-strength solution.

28. (original) The method of claim 27, wherein the ionic solution comprises up to 10 mM salt.

29. (original) The method of claim 1, wherein the solid support is a mineral support or polymer support.

30. (original) The method of claim 29, wherein the mineral support or polymer support is a column comprising silica.

31. (original) The method of claim 29, wherein the mineral or polymer support is a set of beads made of an absorptive polymer.

32. (original) The method of claim 31, wherein the set of beads are collected by centrifugation, filtration, or magnetic capture.

33. (original) The method of claim 30, wherein the silica is glass fiber.

34. (original) The method of claim 1, further comprising passing the lysate through the column by centrifugation or gas pressure.

35. (original) The method of claim 1, further comprising capturing the eluted small RNA molecules.

36. (original) The method of claim 33, wherein the eluted small RNA molecules are captured on a filter and then collected.

37. (original) The method of claim 1, wherein the small RNA molecules are single stranded.

38. (original) The method of claim 1, wherein the small RNA molecules are double stranded.

39. (original) The method of claim 1, wherein the small RNA molecules have at most 100 nucleotides or fewer.

40. (original) The method of claim 39, wherein the small RNA molecules have at most 70 nucleotides or fewer.

41. (original) The method of claim 40, wherein the small RNA molecules have at most 30 nucleotides or fewer.

42. (currently amended) A method for isolating miRNA or siRNA from a sample comprising:

- obtaining a sample having miRNA or siRNA;
- adding an alcohol solution to the sample;
- adding an extraction solution to the sample;

~~[[c]]d~~) applying the sample to a mineral or polymer support; and

~~[[d]]e~~) eluting the siRNA or miRNA from the mineral or polymer support ~~with an ionic solution~~.

43. (original) The method of claim 42, wherein the sample is a cell lysate.

44. (original) The method of claim 43, wherein the cell lysate is produced by adding a lysing solution comprising a chaotropic agent or detergent to cells having miRNA or siRNA.

45. (original) The method of claim 42, wherein the eluted sample is enriched at least about 10-fold by mass for miRNA or siRNA.

46. (currently amended) A method for isolating miRNA molecules from a sample comprising:

- a) adding an alcohol solution to the sample;
- b) applying the sample to a mineral or polymer support;
- c) eluting miRNA molecules from the support ~~with an ionic solution~~; and
- d) using or characterizing the miRNA molecules.

47. (original) The method of claim 46, wherein the sample is a cell lysate.

48. (currently amended) A method for isolating small RNA molecules from a sample comprising:

- a) lysing cells in the sample with a lysing solution comprising guanidinium, wherein a lysate with a concentration of at least about 1 M guanidinium is produced;
- b) extracting small RNA molecules from the lysate with an extraction solution comprising phenol;
- c) adding to the lysate an alcohol solution for form a lysate/alcohol mixture, wherein the concentration of alcohol in the mixture is between about 35% to about 70%;
- d) applying the lysate/alcohol mixture to a mineral or polymer support;
- e) eluting the small RNA molecules from the mineral or polymer support ~~with an ionic solution~~;
- f) capturing the small RNA molecules; and
- g) using the isolated small RNA molecules.

49. (original) A kit for isolating small RNA molecules comprising:

- a) acid phenol-chloroform;
- b) lysis/binding buffer;
- c) homogenate additive;
- d) wash solution;

- e) elution solution; and
- f) filter cartridges.

50. (currently amended) A method for isolating small RNA molecules from a sample comprising:

- a) lysing cells in a lysing solution to produce a lysate;
- b) extracting small RNA molecules from the lysate with an extraction solution comprising phenol;
- c) adding to the lysate an alcohol solution to form a lysate/alcohol mixture;
- d) applying the lysate/alcohol mixture to a first solid support;
- e) collecting flow-through lysate/alcohol mixture;
- f) adding to the flow-through lysate/alcohol mixture an alcohol solution;
- g) applying the lysate/alcohol mixture to a second solid support; and
- h) eluting small RNA molecules from the solid support ~~with an ionic solution~~.

51. (original) The method of claim 50, wherein the lysate/alcohol mixture applied to the first solid support is between about 20% to about 35% alcohol.

52. (original) The method of claim 50, wherein the lysate/alcohol mixture applied to the second solid support is between about 35% to about 70% alcohol.

53. (original) The method of claim 50, further comprising using or characterizing the small RNA molecules.

54. (new) The method of claim 42, wherein elution is with an ionic solution.

55. (new) The method of claim 46, wherein elution is with an ionic solution.

56. (new) The method of claim 48, wherein elution is with an ionic solution.

57. (new) The method of claim 50, wherein elution is with an ionic solution.